

Overview and Summary of Changes made in the Harmonization of OPPTS Toxicology Guidelines with OECD Guidelines.

The major focus of this project has been to harmonize the Toxicology guidelines between the Office of Pollution Prevention and Toxics (OPPT) and the Office of Pesticide Programs (OPP)) within EPA and with the Organization for Economic Cooperation and Development (OECD) guidelines. In addition, OPPTS has made changes to update these guidelines.

Guidelines with major revisions (e.g., developmental toxicity, reproductive effects, dermal absorption and immunotoxicity) have undergone separate peer review. Policy statements, such as the OPP interim policy on inhalation studies and analyses done in the course of the OPP rejection rate analysis, have been incorporated into the revised guidelines in order to provide a consistent presentation of our current positions with respect to the conduct of these studies.

Changes and/or additions were also made for clarification and to ensure uniformity among guidelines within a duration of exposure, (e.g., between the acute guidelines); and among guidelines for a specific route of exposure, (e.g. inhalation guidelines or sections of chronic guidelines).

What follows is a summary of the major changes made in the guidelines. For the subchronic systemic toxicity test guidelines (oral, dermal, and inhalation guidelines) and the chronic toxicity test guidelines, detailed tables of changes are attached at Tables 1-6. Pivotal changes in the subchronic and chronic toxicity and carcinogenicity guidelines are included at Table 7. Finally, a summary is provided of key changes in the guidelines for reproductive effects and developmental toxicity testing.

This has been a major undertaking involving many scientists from both OPPT and OPP. We believe the major goals have largely been met and hope that these guidelines provide an updated and clearer description of our guideline requirements. There are still some minor differences with current OECD guidelines. Through further review and cooperation in the course of the ongoing revision of OECD guidelines, the Agency hopes to address these differences.

1. Group A - Acute Toxicity Test Guidelines

870.1100 Acute Oral Toxicity

870.1200 Acute Dermal Toxicity

870.1300 Acute Inhalation Toxicity

In general, the acute toxicity guidelines, oral, dermal, and inhalation, have been revised to incorporate 1988 EPA Policy guidance on reductions in the limit dose, the numbers of animals used, and general acceptance of the fixed dose method, as described in OECD Guideline 420; and conditional acceptance of the up and down method, as described by American Society for Testing and Materials.

For the acute dermal study, in order to clarify procedures, certain elements of the application of materials on the skin have been specified including description of the area of application, a constant volume of application, and preference for semi-occlusive dressings.

The acute (and subchronic) inhalation guidelines have been modified to incorporate OPP Interim Policy concerns reviewed by SAP concerning particle size and other changes to increase flexibility in conduct of these studies. While these inhalation guidelines are thus different in some ways from existing OECD guidelines (developed based on the previous EPA guidelines in the early 1980s), OECD is currently working on updating the acute inhalation guidelines, for which Germany has the lead, and we anticipate that there is general consensus that our interim policy and anticipated OECD changes are in the same direction, e.g., greater flexibility on particle size needs, and greater guidance on a variety of issues.

2. Group B - Specific Organ/Tissue Toxicity Test Guidelines

870.2400 Acute eye irritation
870.2500 Acute dermal irritation
870.2600 Skin sensitization

In order to conform to changes in OECD guidelines made in 1987 and 1992, guidelines for dermal and ocular irritation and sensitization studies have been revised to incorporate criteria for when staged testing or reduced numbers of animals may be acceptable, provisions for use of alternative testing sequences, and other changes related to the humane use of animals. For the skin sensitization guideline, the references have also been updated.

3. Group C - Subchronic Toxicity Test Guidelines

870.3100 90-Day Oral Toxicity
870.3200 Repeated Dose Dermal Toxicity 21/28 Days
870.3250 Subchronic Dermal Toxicity 90 Days
870.3465 Subchronic Inhalation Toxicity

The subchronic toxicity guidelines have been modified to include, where appropriate, the changes made to the acute studies of the same exposure route, and in general aspects, to be consistent with one another. Limit doses of 1 g/kg for oral and dermal studies have been specified.

The list of Observations has been slightly clarified and modernized and distinction made between daily cage-side observations and more detailed weekly clinical observations. This has been made consistent with the 1/94 OECD revisions to Guideline 407 which is anticipated to be added to the 90 day and longer duration guidelines as well. Observations in OECD Guideline 407, but not EPA (or current OECD) subchronics, call for these detailed observations prior to exposure and in the 4th week. These may be updated as OECD revises the 90 day guidelines.

Tables for clinical chemistry and histopathology have been added to all these guidelines to aid the reader. There are only minor differences between EPA and OECD guidelines on enzyme measures, with the EPA list currently slightly shorter. For example, EPA guidelines call for platelet count AND a measure clotting potential, while current OECD guidelines say platelets OR a clotting potential measure.

Immunotoxicity endpoints, including spleen (all) and thymus weights (21 day dermal only) and white blood cell enumeration (for conduct when appropriate) have been added.

Brain and spleen weights have been added to EPA guidelines, which are consistent with OECD revised Guideline 407, but adrenal weights have been deleted.

Interim inhalation policy changes are also incorporated into the 90 day inhalation guideline, including particle size specifications, and a limit dose of 2 mg/l has been added to the 90 day inhalation guideline.

There are some minor differences remaining in histopathology of organs recommended, i.e., salivary glands, mammary glands, and eyes required by EPA, listed as only if indicated by OECD.

Additional comparisons are provided in the attached Tables 1-3.

Group C - Subchronic Toxicity Test Guidelines (cont'd.)

870.3700 Prenatal Developmental Toxicity Study
870.3800 Reproduction and Fertility Study

Changes were made to these guidelines during their revision and subsequent to the Scientific Advisory Panel (SAP) meeting in December 1993. See Table 8 for a summary of changes as well as additional public comments obtained during the summer of 1994. Because the changes are extensive, additional SAP review and comment is sought at this time.

4. Group D - Chronic Toxicity Test Guidelines

870.4100 Chronic Toxicity
870.4200 Carcinogenicity
870.4300 Combined Chronic Toxicity/Carcinogenicity

As with the subchronic guidelines, the chronic toxicity and carcinogenicity guidelines have been modified to be consistent with respect to general features of one another. Similarly, changes related to route of exposure have also been made, based on the acute and subchronic, e.g. inhalation, guidelines. Inhalation Guidance derived from the revised Inhalation guidelines has been added. Language has been standardized with respect to many general sections, e.g., observations,

age at testing, as described for the subchronic guidelines. Limit doses of 1 g/kg have also been specified here.

Tables for clinical chemistry and histopathology have been added for clarity. The frequency of hematological, clinical chemistry, and urinalyses have been standardized for rodents, at 6 month intervals, (at 3 months only if effects were seen in subchronic studies), and for non-rodents prior to exposure (1-2 times) and at 3 month intervals thereafter.

Ophthalmological exams have been maintained for rodents (10/sex/dose) and all non-rodents prior to exposure and at termination. This remains a difference from current OECD Chronic, but not OECD subchronic guidelines, which do call for these measures.

In addition to guidance on dermal dosing application procedures, criteria for the adequacy of dermal dosing levels, derived from an OPPT workshop, have been incorporated into the Carcinogenicity Guidelines. The mouse has been specified as the preferred species for studies by this route.

For histopathology, EPA guidelines specify all lungs, livers, kidneys and targets organs be examined, while OECD guidance is more general.

Changes in the specification of dose levels, particularly with respect to the adequacy of the high dose in carcinogenicity studies have not been made; it is anticipated that current efforts underway between OPP, its contractor, ILSI, and OECD will lead to proposal for revisions of those sections.

Further details of the revisions are provided in attached Tables 4-6.

5. Group E - Genetic Toxicity Test Guidelines

870.5100 - 870.5900

The genetic toxicology guidelines which are part of the health effects guideline package for review were first published in the mid-1980s and have not been revised since. They are all in *de facto* complete agreement with the guidelines published by the Organization for Economic Cooperation and Development (OECD) where OECD has comparable guidelines. Some of the guidelines in the health effects review package, e.g., gene mutation in *Aspergillus nidulans*; the visible specific locus test, are unique to OPPTS. These guidelines were subject to extensive peer review when they were first published and have not been revised. They should require no special SAP review or public comment at this time..

OECD is in the process of updating its gene-tox guidelines: Reverse mutation in *Salmonella typhimurium* and reverse mutation in *Escherichia coli* are being combined into 1 guideline; in vitro cytogenetics, in vivo cytogenetics (chromosomal aberrations); mammalian

micronucleus assay; in vitro assay for gene mutation and the sperm cytogenetics assay are being revised and new assay for *in vivo/in vitro* unscheduled DNA synthesis is being developed. These assays are in the final stages of OECD peer review and are being included as part of the health effects guidelines at this time. When the OECD guidelines are adopted, the U.S. EPA will harmonize its guidelines with those of the OECD and the interested public will be informed of the change.

6. Group F - Neurotoxicity Test Guidelines

870.6100 Delayed Neurotoxicity of Organophosphorus Substances following Acute and 28 Day Exposures

870.6200 Neurotoxicity Screening Battery

870.6300 Developmental Neurotoxicity Study

870.6500 Schedule-controlled Operant Behavior

870.6850 Peripheral Nerve Function

870.6855 Neurophysiology: Sensory Evoked Potentials

These guidelines were developed as a joint effort by OPPT and OPP and all but 870.6855 were previously submitted for SAP review prior to initial publication. Thus, 870.6100, 870.6200, 870.6300, 870.6500, and 870.6850 are being republished with no changes. Guideline 870.6855, on sensory evoked potentials was developed and has been peer reviewed in OPPTS and outside EPA. It should be reviewed by the SAP with these revised guidelines.

870.6100, on Delayed Neurotoxicity of Organophosphates, has been harmonized with the revised OECD Guidelines 418 and 419. There are currently no other separate OECD Neurotoxicity Test Guidelines, but there are a number of activities in progress through the OECD:

Changes were made to OECD Guideline 407, 28-day Subchronic Toxicity Study, to include some explicit observations related to neurotoxicity;

A draft OECD test guideline similar to the 870.6200 Neurotoxicity Screening Battery is under review by member countries;

Based on the recommendations of OECD expert groups on Neurotoxicity and Developmental Toxicity, it is expected that a guideline for Developmental Neurotoxicity will be considered by OECD for possible development.

7. Group G - Special Studies Test Guidelines

870.7485 Metabolism and Pharmacokinetics

870.7600 Dermal Penetration
870.7800 Immunotoxicity

The revised EPA Metabolism and Pharmacokinetic guideline is currently out for public comment subsequent to a Federal Register Notice and will be subject to SAP review as part of this package. EPA is taking the lead in this area.

The immunotoxicity test guideline is new and has been developed by EPA subsequent to review by the SAP in December 1993 of a document setting forth the parameters for immunotoxicity testing.

The Dermal Penetration guideline has previously been reviewed by the public and SAP, revised, and published through the National Technical Information Service (NTIS). Currently, OECD has a draft guideline in this area under review. Harmonization will be achieved through our involvement in that process. Thus, further extensive review of this guideline is not requested at this time.

8. Group H - Health Effects Chemical-Specific Test Guidelines

Group H (870.8223 - 870.8800) contains chemical-specific test guidelines developed by OPPT for individual test rules. In addition 870.1350, Acute inhalation toxicity with histopathology, was developed in the recent Hazardous Air Pollutant test rule. OPP does not anticipate applying these test guidelines generically. Therefore, peer review and comment are not being requested. Rather, these test guidelines are published for completeness.

Table 1. 21/28 REPEATED DOSE DERMAL TOXICITY (OPP 82-2; OECD 410)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|-------------------------------------|---|--|---|
| Principle of Test Method | Describes the study | <u>Deletion</u> | Repetitive of the study procedures. Harmonize with other guidelines. |
| Preparations | Describes study protocol | <u>Deletion</u> | Not appropriate here. Details included under "Test Procedures" and provides specific requirements for shaving, treatment area, dosing volume, and application procedures. |
| Selection of Species | The adult rat, rabbit or guinea pigs may be used. Specifies weight ranges for these species. | <u>Deletion:</u> Testing should start with young healthy animals as soon as possible after weaning and acclimatization. Dosing should generally begin no later than 8 weeks of age. Weight variation should not exceed ±20 % of the mean weight for each sex/species. | Harmonize with other guidelines. Dosing age same as other guidelines. Age/weight specification are same as the other guidelines. |
| Number and Sex | At least 10 animals (5 males and 5 females) at each dose level. Also, a satellite group of 10 animals (5/sex) | <u>Revision:</u> 20 animals (10 males and 10 females at each dose level. Satellite groups of 20 animals (10/sex) | Harmonize with OPP and with other subchronic studies. |
| Physical Observation | Provides only a "general" description of procedure to be used. | <u>Revision:</u> Provides a more detailed procedures (e.g., includes observations for neuro toxicity. | Revisions necessitated by to differentiate between daily cage-side obs. vs. weekly clinical obs. |
| None | None | <u>Addition:</u> A battery of immunotoxicity screen included. | Addition necessitated by the need to look for immunotoxic effects. |
| Ophthalmology | Not indicated in the guideline. | <u>Addition:</u> Performed on all animals pre-initiation and on all high dose and control animals at termination. | Required in the 90-day dermal study. Also, harmonize with OPP. |
| Necropsy | No spleen weight | <u>Addition:</u> Spleen weight. | Because of the immunotoxicity screen. |
| Histopathology | Does not specify target organs, liver, lungs and kidneys of all animals | <u>Addition:</u> Includes target organs, liver, lungs and kidneys on all animals. | Harmonize with OPP & OTS and with the other subchronic guidelines |
| Study Reports: Presentation of data | Inadequate. | <u>Revision:</u> Provides data/format requirements for test reports, specifically for, test substance, test system, test environment, and test results. | Harmonize with OPP & OTS and other guidelines (such chronic, carcinogenicity, combined chronic/carcinogenicity) |

Table 2. 90-DAY INHALATION TOXICITY (OPP 82-4; OECD 413)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|--------------------------|---|--|---|
| Definitions | Definition of subchronic inhalation only. | <u>Addition:</u> In addition to definition of subchronic inhalation also includes definition of concentration, NOEL, aerodynamic diameter, MMAD, and inhalation diameter. | Revision needed. |
| Principle of Test Method | Describes the study | <u>Deletion</u> | Repetitive of the study procedures. Harmonize with other guidelines. |
| Preparations | Describes study protocol | <u>Deletion</u> | Not appropriate here. Details included under "Test Procedures" and provides specific requirements for shaving, treatment area, dosing volume, and application procedures. |
| Selection of Species | Preferred species is rats, and young healthy animals should be employed. | <u>Revision:</u> Testing should start with young healthy animals as soon as possible after weaning and acclimatization. Dosing should generally begin no later than 8 weeks of age. Weight variation should not exceed $\pm 20\%$ of the mean weight for each sex/species. | Harmonize with other guidelines. Dosing age same as other guidelines. Age/weight specification are same as the other guidelines. |
| Number/Sex | Only rats (10/sex/dose) mentioned. | If another mammalian species is selected (dogs, rabbits, or non-human primates) at least 8 animals (4/sex) shall be used. | To facilitate inhalation studies in other species. |
| Equipment | Air flow of 12 to 15 air changes/hour. | <u>Revision:</u> A minimum of air flow of 10 air changes/hour also includes other exposure specifications. | Based on OPP's "Interim Policies" for inhalation studies. |
| Exposure Concentrations | In the low and intermediate groups and in the controls the incidence of fatalities should be low. | <u>Revision:</u> The lowest concentration should produce no evidence of toxicity. | Harmonize with the dose-response definitions/specifications for the high, intermediate and low dose levels. |

Table 2. 90-DAY INHALATION TOXICITY (OPP 82-4; OECD 413) (Continued)

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|-----------------------|--|--|---|
| Physical Measurements | The rate of air flow, preferably, should be monitored continuously. During exposure the actual concentrations should be held as constant as practicable. | The rate of air flow shall be monitored continuously <u>but</u> recorded at least every 30 minutes....includes other specifications. | Based on OPPs "Interim Policies for inhalation studies. |
| | No specifications of particle size. Temperature and humidity (preferably continuously). | The MMAD particle size range should be between 1-3 microns...also other specifications. Temperature and humidity shall be monitored continuously but shall be recorded at least every 30 minutes. | |
| Physical Observation | Provides only a "general" description of procedure to be used. | <u>Revision:</u> Provides a more detailed procedures (e.g., includes observations for neurotoxicity. | Revisions necessitated to differentiate between daily cage-side obs. vs. weekly clinical obs. |
| Clinical Pathology | Hematology and clinical chemistry measure in rats at termination. | Addition: For rodents at termination. For non-rodents, once prior to initiation, at monthly intervals or midway thru the test period and at termination. | Includes rodents and non-rodents. |
| None | None | <u>Addition:</u> A battery of immunotoxicity screen included. | Includes rodents and non-rodents. |
| Ophthalmology | At termination, preferably in all animals, but at least in the high dose and control groups. | <u>Revision:</u> At termination performed on all animals in the high dose and control groups. | Harmonize with OPP & OTS and other guidelines. |
| Necropsy | No spleen weight | <u>Addition:</u> Spleen weight. | Because of the immunotoxicity screen. |
| Histopathology | Does not specify target organs, liver, lungs and kidneys of all animals | <u>Addition:</u> Includes target organs, liver, lungs and kidneys on all animals. | Harmonize with OPP & OTS and with the other subchronic guidelines |
| Presentation of data | Inadequate requirements. | <u>Revision:</u> Provides data/format requirements for test reports, specifically for, test substance, test system, test environment, and test results. | Harmonize with OPP & OTS and other guidelines (such chronic, carcinogenicity, combined chronic/carcinogenicity) |

Table 3. 90-DAY ORAL TOXICITY (OPP 82-1; OECD-408 Rodent & 409-Nonrodent)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|--------------|-------------------|-----------------|--------------------|
|--------------|-------------------|-----------------|--------------------|

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|-------------------------------------|---|---|---|
| Principle of Test Method | Describes the study | <u>Deletion</u> | Repetitive of the study procedures. Harmonize with other guidelines. |
| Preparations | Describes study protocol | <u>Deletion</u> | Not appropriate here. Details are included under "Test Procedures". |
| Selection of Species | Provides animal species and weight ranges. For rodents, dosing should begin ideally before the rats are 6 , and in any case not more than 8 weeks old. Non-rodents, 4-6 months, no later than 9 months. | <u>Deletion:</u> Dosing of rodents should generally begin no later than 8 weeks of age. Non-rodents, 4-6 months, no later than 9 months. | Harmonize with other guidelines Age/weight specification are same as the other guidelines. |
| Physical Observation | Provides only a "general" description of procedure to be used. | <u>Revision:</u> Provides a more detailed procedures (e.g., includes observations for neurotoxicity. | Revisions necessitated by to differentiate between daily cage-side obs. vs. weekly clinical obs. |
| Ophthalmology | At termination preferably on all animals but at least in the high dose and control groups. | <u>Revision:</u> At termination on all high dose and control groups. | Harmonize with OPP & OTS and with other guidelines. |
| None | None | <u>Addition:</u> A battery of immuno-toxicity screen included | Addition necessitated by the need to look for immunotoxic effects (recommended by SAP) |
| Necropsy | No spleen weight | <u>Addition:</u> Spleen weight. | Because of the immunotoxicity screen. |
| Histopathology | Does not specify target organs, liver, lungs and kidneys of all animals | <u>Additon:</u> Includes target organs, liver, lungs and kidneys on all animals. | Harmonize with OPP & OTS and with the other subchronic guidelines |
| Study Reports: Presentation of data | Inadequate. | <u>Revision:</u> Provides data/format requirements for test reports, specifically for, test substance, test system, test environment, and test results. | Harmonize with OPP & OTS and other guidelines (such chronic, carcinogenicity, combined chronic/carcinogenicity) |

Table 4. CHRONIC TOXICITY (OPP 83-1 a,b; OECD 452)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|--------------|-------------------|---|--|
| DEFINITIONS | None | Addition. Definitions of chronic toxicity, dose, NOEL, target organ, and cumulative toxicity added. | Harmonize with OPP & OTS and with other (subchronic, carcinogenicity, combo) guidelines. |

| Preliminary/ Additional Test | Requires the test chemical's physical and chemical properties | <u>Deletion</u> | Harmonize with OPP & OTS and also not appropriate here since this requirement is covered elsewhere. |
|--|--|---|---|
| Age/Maturity | Dosing to begin during the rapid growth phase, soon after weaning and acclimation. No specification for body weight distribution. | Addition: Dosing of rodents no later than 8 weeks; non-rodents 4-6 months, no later than 9 months. Weight variation should not exceed ±20% of the mean for each sex. | Harmonize with OPP & OTS, both indicate age/weight specification. |
| Housing and Feed Conditions | In general provides more detail than OTS and OPP. | <u>Deletion.</u> | Basic requirements of housing, feed, environmental conditions states. Details can be obtained from GLP. |
| Compound Purity, Reference, and Vehicle | Prerequisite information must be known about the chemical prior to testing and the use of water in inhalation studies implied. | <u>Revision:</u> Section revised to include information on control and test substances (i.e., type of vehicle, lot numbers, purity, analysis of the dosing material). | Section needed to be revised |
| Exposure conditions: Dose selection, Route, and Duration. | Separated by sections and information is repetitive and overlaps the requirements described under "5 Experimental Techniques" | <u>Revision:</u> Dose selection defines the three doses to be used. Also, the highest dose tested need not exceed 1000 mg/kg/day <u>Revision:</u> Route, schedule, and duration is covered under "Administration of test substance". | Harmonize with OTS & OPP and with the other guidelines (subchronic, carcinogenicity, combined chronic tox/carcinogenicity). |
| Experimental Techniques | Describes the experimental procedures to be used by the oral, dermal, and inhalation routes. | <u>Revision:</u> Includes more details on oral studies; dermal studies (application procedures, etc.); inhalation studies (chamber conditions, concentration, particle size, temperature/ humidity, etc.); duration of 12 months for all routes. | Revisions necessitated by rejection rate study for shaving/dosing area/volume, etc. for dermal studies and OPP's interim policies for inhalation studies. |
| Physical Observation | Provides only a "general" description of procedure to be used. | <u>Revision:</u> Provides a more detailed procedures (e.g., includes observations for neurotoxicity). | Revisions necessitated by to differentiate between daily cage-side obs. vs. weekly clinical obs. |
| Hematology | Performed at 3 months, 6 months, at 6-months interval thereafter & termination from 10 rats/sex and all non-rodents. | <u>Revision:</u> Performed at approximately at 6 months intervals in rodents (10/sex/dose). Also, if effects are seen in the 90-day, then testing should be done at 3 months. In all non-rodents, once/twice prior to initiation, at 3 month intervals and termination. | Interval reduced; the 3 month interval not needed since data can be obtained from 90-day studies. |
| Clinical chemistry | Performed at approximately 6 month intervals. | <u>Revision:</u> At same intervals as hematology on same no.of animals. | Same reason as above |

Table 4. CHRONIC TOXICITY (OPP 83-1 a,b; OECD 452) (Continued)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|--------------|-------------------|-----------------|--------------------|
|--------------|-------------------|-----------------|--------------------|

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|--|--|--|---|
| Urinalysis | If needed, then same interval as hematology | <u>Revision:</u> At same intervals as hematology & clinical chemistry on same number of animals. | Data obtained at same intervals and no.of animals for all three parameters. |
| None | None | <u>Addition:</u> A battery of immunotoxicity screen added. | Addition necessitated by the need to look for immunotoxic effects (recommended by SAP) |
| Ophthalmology | Not indicated in the guideline | Performed on all animals pre-initiation and on 10/sex in the high dose & controls at termination. | Harmonize with OPP & OTS |
| Necropsy | No spleen weights | <u>Addition:</u> Spleen weights | Added due to immunotoxicity screen |
| Histopathology | Does not specify target organs, liver, lungs, and kidneys of all animals | <u>Addition:</u> Includes the target organs, lungs, liver and kidneys on all animals | Harmonize with OPP & OTS |
| Quality Control | Provides too much detail on QA requirements which are not necessary in this section. | <u>Revisions:</u> Just states that the study must be conducted in compliance with GLP regulations of EPA and OECD principles | The burden is on the laboratory/sponsor to follow QA procedures. No need to go into this detail here. They are covered under GLP. |
| Statistical Analysis | Not indicated in the guideline | <u>Addition:</u> All observed results (quantitative and qualitative) evaluated by appropriate statistical methods. | Harmonize with OPP & OTS and other guidelines (subchronic, carcinogenicity, combined chronic/carcinogenicity) |
| Data Evaluation | Not indicated in the guideline | <u>Addition:</u> Provides methods for data evaluation. | Harmonize with OPP & OTS and other guidelines (subchronic, carcinogenicity, combined chronic/carcinogenicity) |
| Study Reports: Presentation of Data | Not indicated in the guideline | <u>Addition:</u> Provides data/format requirements for test reports, specifically for, test substance, test system, test environment, and test results. Also certain requirements for reporting of inhalation studies. | Harmonize with OPP & OTS and other guidelines (subchronic, carcinogenicity, combined chronic/carcinogenicity) |

Table 5. CARCINOGENICITY (OPP 83-2a,b; OECD 451)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|---|--|--|---|
| Definitions | None | <u>Addition:</u> Definitions of carcinogenicity, dose, NOEL, target organ, and cumulative toxicity added. | Harmonize with OPP & OTS and with other guidelines (subchronic, chronic, combined chronic/carcinogenicity) |
| Preliminary/ Additional Test | Requires the test chemical's physical and chemical properties | <u>Deletion</u> | Harmonize with OPP & OTS and also not appropriate here since this requirement is covered elsewhere. |
| Species/Strain | Non-specific; covers all laboratory species. | <u>Addition:</u> Rats and mice are species of choice and for mice is the first test species for skin carcinogenesis. | Guidance for skin carcinogenicity studies. Harmonize with the combined chronic tox/carcinogenicity study guideline. |
| Age/Maturity | Dosing to begin during the rapid growth phase, soon after weaning and acclimation. No specification for body weight distribution. | <u>Addition:</u> Dosing of rodents no later than 8 weeks; Non-rodents 4-6 months, no later than 9 months. <u>Weight variation should not exceed ±20% of the mean for each sex</u> | Harmonize with OPP & OTS, both indicate age/weight specification. Also, with other guidelines (subchronic, chronic, combined chronic/carcinogenicity). |
| Housing and Feed Conditions | In general provides more detail than OTS and OPP. | <u>Deletion</u> | Basic requirements of housing, feed, environmental conditions states. Details can be obtained from GLP. |
| Compound Purity, Reference, and Vehicle | Prerequisite information must be known about the chemical prior to testing and the use of water in inhalation studies implied. | <u>Revision:</u> Section revised to include information on control and test substances (i.e., type of vehicle, lot numbers, purity, analysis of the dosing material). | Section needed to be revised |
| Exposure conditions: Dose selection, Route, and Schedule. | Separated by sections and information is repetitive and overlaps the requirements described under "Experimental Techniques" | <u>Revision:</u> Dose selection defines the three doses to be used. Also, the highest concentration tested need not exceed 1000 mg/kg/day <u>Addition:</u> Criteria for selecting dose levels for skin carcinogenesis studies. <u>Revision:</u> The route, schedule, and duration is covered under "Administration of test substance". | Harmonize with OTS & OPP and also with the other guidelines (subchronic, chronic, combined chronic/carcinogenicity) No guidance was provided for selecting dose levels for skin carcinogenesis studies. Harmonize with OTS & OPP and also with the other guidelines |
| Experimental Techniques | Describes the experimental procedures to be used for the oral, dermal, and inhalation routes. | <u>Revision:</u> Includes shaving, dosing area, dosing volume, and application techniques etc. for dermal studies and OPP's interim policies such as chamber conditions, exposure specifications, and physical measurements etc. for inhalation studies. | Necessitated by rejection rate study. |

Table 5. CARCINOGENICITY (OPP 83-2a,b; OECD 451) (Continued)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|--------------|-------------------|-----------------|--------------------|
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| Observation Period | None specified | Observation period shall not be less than 24 months for rats and 18 months for mice, and ordinarily not longer than 30 months for rats and 24 months for mice. For longer periods, and where any other species are used, consultation with Agency in regard to the duration of the study is advised. | Harmonize with OTS & OPP and with the combined chronic toxicity/carcinogenicity study guideline. |
| Physical Observation | Provides only a "general" description of procedures to be used. | <u>Revision:</u> Provides more detailed procedures (e.g., includes observations for neurotoxicity) | Revisions necessitated to differentiate between daily cage-side obs. vs. weekly clinical obs. |
| None | None | <u>Addition:</u> A battery of immunotoxicity screen at termination is added. | Addition necessitated by the need to look for immunotoxic effects (recommended by SAP) |
| Necropsy | No spleen weights | <u>Addition:</u> Spleen weights | Added due to immunotoxicity screen |
| Histopathology | Does not specify target organs, liver, lungs, and kidneys of all animals | <u>Addition:</u> Includes the target organs, lungs, liver and kidneys on all animals | Harmonize with OPP & OTS |
| Quality Control | Provides too much detail on QA requirements which are not necessary in this section. | <u>Revision:</u> Just states that the study must be conducted in compliance with GLP regulations of EPA and OECD principles | The burden is on the laboratory/sponsor to follow QA procedures. No need to go into this detail here as they are covered under GLP. |
| Statistical Analysis | Not indicated in the guideline | <u>Addition:</u> All observed results (quantitative and qualitative) evaluated by appropriate statistical methods. | Harmonize with OPP & OTS and with other guidelines (subchronic, chronic, combined chronic/carcinogenicity) |
| Data Evaluation | Indicates that early termination of the study is acceptable if the number of survival in the lower doses or control group reaches 25%. | <u>Revision:</u> Survival should not fall below 50% at 15 months for mice and 18 months for rats and below 25% at termination (18 months for mice and 24 months for rats). | Harmonize with OPP & OTS and with other guidelines (subchronic, chronic, combined chronic/carcinogenicity) |
| Study Reports: Presentation of data | Not indicated in the guideline | <u>Addition:</u> Provides data/format requirements for test reports, specifically for, test substance, test system, test environment, and test results. Also certain requirements for reporting of inhalation studies. | Harmonize with OPP & OTS and with other guidelines (subchronic, chronic, combined chronic/carcinogenicity) |

Table 6. COMBINED CHRONIC TOX/CARCINOGENICITY (OPP 83-5; OECD 453)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|--|--|--|--|
| Definitions | None | <u>ADDITION:</u> Definitions of chronic toxicity, carcinogenicity, dose, NOEL, target organ, and cumulative toxicity added. | Harmonize with OPP & OTS and with other guidelines (subchronic, chronic, carcinogenicity) |
| Preliminary/ Additional Test | Requirements for satellite group, other and the test chemical's physical and chemical properties | <u>Deletion</u> | Harmonize with OPP & OTS and also not appropriate here since this requirements are covered elsewhere. |
| Species/Strain | Non-specific: mice and rats used for carcinogenicity and rats used for the combined. | <u>Revision:</u> Rat is preferred for the combined study via the oral and inhalation routes while mice are preferred for the dermal route. | Harmonize with OPP & OTS and with the carcinogenicity test guideline. |
| Age/Maturity | Dosing to begin during the rapid growth phase, soon after weaning and acclimation. No specification for body weight distribution. | <u>Addition:</u> Dosing of rodents no later than 8 weeks; Non-rodents 4-6 months, no later than 9 months. <u>Weight variation should not exceed ±20% of the mean for each sex</u> | Harmonize with OPP & OTS, both indicate age/weight specification. Also, with other guidelines (subchronic, chronic, carcinogenicity). |
| Housing and Feed Conditions | In general provides more detail than OTS and OPP. | <u>Deletion.</u> | Basic requirements of housing, feed, environmental conditions states. Details can be obtained from GLP. |
| Compound Purity, Reference, and Vehicle | Prerequisite information must be known about the chemical prior to testing and the use of water in inhalation studies implied. | <u>Revision:</u> Section revised to include information on control and test substances (i.e., type of vehicle, lot numbers, purity, analysis of the dosing material). | Section needed to be revised |
| Exposure conditions: Dose selection, Route, and Schedule /Duration. | Separated by sections and information is repetitive and overlaps the requirements described under " Experimental Techniques" | <u>Revision:</u> Dose selection defines the three doses to be used. Also, the highest concentration tested need not exceed 1000 mg/kg/day <u>Addition:</u> Criteria for selecting dose levels for skin carcinogenesis studies. | Harmonize with OTS & OPP and also with the other guidelines (subchronic, chronic, carcinogenicity). No guidance was provided for selecting dose levels for skin carcinogenesis studies. |
| Experimental Techniques | Describes the experimental procedures to be used for the oral, dermal and inhalation routes. | <u>Revision:</u> The route, schedule, and duration is covered under "Administration of test substance". | Harmonize with OTS & OPP and also with the other guidelines |
| | | <u>Revision:</u> Includes shaving, dosing area, dosing volume, and application techniques etc. for dermal studies and OPP's interim policies such as chamber conditions, exposure specifications, and physical measurements etc. for inhalation studies. | Necessitated by the rejection rate study. |

Table 6. COMBINED CHRONIC TOXICITY/CARCINOGENICITY (OPP 83-5; OECD 453) (Continued)

| OECD SECTION | EXISTING CRITERIA | PROPOSED CHANGE | REASONS FOR CHANGE |
|---------------------|--------------------------|------------------------|---------------------------|
|---------------------|--------------------------|------------------------|---------------------------|

| | | | |
|-------------------------------------|---|--|--|
| Observation period | Not in the guideline | Observation period shall not be less than 24 months for rats and 18 months for mice, and ordinarily not longer than 30 months for rats and 24 months for mice. For longer periods, and where any other species are used, consultation with Agency in regard to the duration of the study is advised. | Harmonize with OPP & OTS and with the carcinogenicity study guideline. |
| Physical Observation | Provides only a "general" description of procedures to be used. | Revision: Provides more detailed procedures (e.g., includes observations for neurotoxicity) | Revisions necessitated to differentiate between daily cage-side obs. vs. weekly clinical obs. |
| Hematology | Performed at 3 months, 6 months, at 6-month intervals thereafter and at termination on 20 rats/sex of all groups. | Revision: Performed at approximately 6 month intervals and at termination from 10/sex/group. If effects are seen in the subchronic study, testing should be done at 3 months. | Interval reduced; 3 month not needed since data can be obtained from 90-day studies. |
| Clinical chemistry | Performed at approximately 6 month intervals, and at termination from 10 rats/sex/group. | Revision: Performed at same intervals as hematology on same number of animals. | Same reason as above |
| Urinalysis | If needed, then at same interval as hematology | Revision: Performed at same interval as hematology and clinical chemistry on same number of animals. | Data obtained from all three parameters at same intervals on same number of animals. |
| Ophthalmology | Not in the guideline. | Performed on all animals pre-initiation and on 10/sex in the high dose & controls at termination. | Harmonize with Opp & OTS. |
| None | None | Addition: A battery of immunotoxicity screen at termination is added. | Addition necessitated by the need to look for immunotoxic effects (recommended by SAP). |
| Necropsy | No spleen weights | Addition: Spleen weights | Added due to immunotoxicity screen |
| Histopathology | Does not specify target organs, liver, lungs, and kidneys of all animals | Addition: Includes the target organs, lungs, liver and kidneys on all animals | Harmonize with OPP & OTS and with the other guidelines (subchronic, chronic, carcinogenicity). |
| Quality Control | Provides too much details on QA requirements which are not necessary for the intended purpose. | Revision: Just states that the study must be conducted in compliance with GLP regulations of EPA and OECD principles | The burden is on the laboratory/sponsor to follow QA procedure. No need to go into details that are covered under GLP. |
| Statistical Analysis | Not indicated in the guideline | Addition: All observed results (quantitative and qualitative) evaluated by appropriate statistical methods. | Harmonize with OPP & OTS and with other guidelines. |
| Data Evaluation | Survival in each group is no less than 50% at 18 months for mice and hamsters and at 24 months for rats. | Revision: Survival should not fall below 50% at 15 months for mice and 18 months for rats and below 25% at termination (18 months for mice and 24 months for rats). | Harmonize with OPP & OTS and with the carcinogenicity guideline. |
| Study Reports: Presentation of data | Not indicated in the guideline | Addition: Provides data/format requirements for test reports, specifically for, test substance, test system, test environment, and test results. Also certain requirements for reporting of inhalation studies. | Harmonize with OPP & OTS and with other guidelines. |

Table 7. SUMMARY OF PIVOTAL CHANGES IN SELECTED CRITERIA RESULTING FROM HARMONIZATION-APPLIES TO ALL TOXICOLOGY TEST GUIDELINES.

| CRITERIA | CURRENT OECD | PROPOSED CHANGE | REASON FOR CHANGES |
|------------------------------------|---|---|--|
| Age/Weight | As soon as possible after weaning, Rodents before 6 weeks, not later than 8 weeks. Non-rodents, 4-6 months, not later than 9 months. No weight ranges specified. | Testing should be started with young healthy animals as soon as possible after weaning and acclimatization Dosing should generally begin no later than 8 week of age for rodents. For non-rodents dosing should generally begin at 4-6 months, no later than 9 months. The weight variation of animals used shall not exceed ±20% of the mean weight for each sex. | Harmonize with OPP & OTS. |
| Husbandry | Consists of too much detail on housing, environment, feed and water. | Provides the basic requirements for these elements. | Not needed since these factors are covered under GLP requirements and must be followed. |
| Dose Level & Dose Level Selection. | Definition of high, intermediate and low doses are different from OPP and OTS. | The highest dose need not exceed 1000 mg/kg/day The intermediate dose level(s) should be spaced to produce a gradation of toxic effects. The lowest dose should not produce any evidence of toxic effects. No guidance for the selection of dose levels for skin carcinogenicity studies. | Harmonize with OPP & OTS and also definitions were revised. Based on the dermal carcinogenesis workshop |

Table 7. SUMMARY OF PIVOTAL CHANGES IN SELECTED CRITERIA RESULTING FROM HARMONIZATION - APPLIES TO All TOXICOLOGY TEST GUIDELINES (Continued).

| CRITERIA | CURRENT OECD | PROPOSED CHANGE | REASON FOR CHANGES |
|----------------------------------|--|--|--|
| Administration of Test Substance | The route, schedule, duration, and experimental procedures are discussed under separate headings and are often repetitive. | <p>Under one heading: Clarifies the route, schedule of dosing, and duration of studies and also includes specifics for types of studies.</p> <p>Dermal studies - includes details on shaving, treatment area, dosing volume, and application procedures.</p> <p>Inhalation studies - incorporates the interim policies and includes particulars on chamber conditions, exposure specification, and physical (concentration/particle size) measurements.</p> <p>Schedule - Ideal dosing is on a 7-day per week basis, however, based on practical considerations, dosing for 5-days per week basis is acceptable.</p> <p>Duration - For subchronic studies, the dosing is for 90 days. For chronic toxicity, the dosing is for 12 months (rodents and non-rodents). For carcinogenicity and the combined chronic toxicity/carcinogenicity studies, dosing is 18 months for mice and 24 months for rats.</p> | <p>Needed revision</p> <p>Based on the Rejection Rate study</p> <p>OPPs "Interim Policies" for inhalation studies.</p> <p>No change; same as OECDs</p> <p>Harmonize with OPP & OTS. Clarifies the duration, specially, the chronic vs. the combined/ carcinogenicity</p> |
| Observation Period | Not included in the guideline | In the carcinogenicity and the combined chronic tox/carcinogenicity studies: observation period shall not be less than 24 months for rats and 18 months for mice, and ordinarily not longer than 30 months for rats and 24 months for mice. For longer periods, and where any other species are used, consultation with Agency in regard to the duration of the study is advised. | Harmonize with OPP & OTS |

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|--------------|---|---|---|
| Observations | Provides only a general description of procedures for observations. | Revised and includes criteria for observation of neurotoxicity. | Revisions needed to differentiate twice daily cage side observations vs. weekly clinical observations |
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Table 7. SUMMARY OF PIVOTAL CHANGES IN SELECTED CRITERIA RESULTING FROM HARMONIZATION - APPLIES TO ALL TOXICOLOGY TEST GUIDELINES (Continued).

| CRITERIA | CURRENT OECD | PROPOSED CHANGE | REASON FOR CHANGES |
|--|--|---|---|
| Clinical Pathology | In subchronics: at termination. | No change; at termination in the 90-day studies. | Harmonize with OPP & OTS. |
| No changes any of the existing hematology, clinical chemistry and urinalysis parameters. | In the chronic and the combined chronic/carcinogenicity studies: Hematology at 3 months, 6 months, at 6-month intervals and at termination. Clinical chemistry at 6-month interval and at termination Urinalysis, same as hematology. | Hematology, clinical chemistry and urinalysis performed at 6-months, at approximately 6-month intervals, and at termination in 10 animals/sex/group. If effects are seen in the subchronic studies, then testing is also done at 3-months. | All 3 tests conducted at the same intervals. 3-month hematology data can be obtained from the 90-day study. If effects seen, then a 3-month is purposeful. |
| Ophthalmology | In the 90 day studies: prior to initiation and termination on all animals, but preferably, in the high dose and controls. Not included in the chronic and the combined chronic/carcinogenicity study. | In all the 90-day studies: on all animals pre-initiation and on all animals in the high dose & control groups. In the chronic study, and the combined chronic/carcinogenicity study: prior to initiation in all animals. At termination, on 10/sex/group in rodents and on all nonrodents from the high dose and control groups. | Harmonize with OPP & OTS |
| Immunotoxicity | Not included in the guideline | Included in all the guidelines an immunotoxicity screen: In rodents (10/sex/dose) at the end of the study and include Total T-, Total B-, Total T-helper, T-suppressor/cytotoxic and Natural Killer (NK) cell populations. | To evaluate immunotoxicity |
| Necropsy | Not included in the guideline | Added spleen weight | Due to immunotox.screen. |
| Histopathology | Not included in the guideline | Requires histopathology of the lungs, liver, kidneys, and target organs on all animals. | Harmonize with OPP & OTS |
| Data evaluation | In carcinogenicity: negative study acceptable if survival is 25% in control or low dose. In the combined: 50% at termination (18 months for mice and 24 months for rats) | In the carcinogenicity and the combined chronic toxicity/carcinogenicity studies, survival should not fall below 50% at 15 months for mice and 18 months for rats. Survival should not fall below 25% at termination (18 months for mice and 24 months for rats). | Harmonize with OPP & OTS. |

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|--------------|-----------------------|--|--------------------------|
| Study Report | Not in the guideline. | Provides data/format requirements for study reports. | Harmonize with OPP & OTS |
|--------------|-----------------------|--|--------------------------|

Table 8—Summary of Changes for Developmental Toxicity and Reproduction Studies**A. Major changes to the developmental toxicity testing guideline:****Maternal endpoints:**

- Dosing from implantation through termination
- Periodic dosage adjustment throughout administration
- Require 20 pregnant rabbits per dose group

Fetal endpoints:

- Skeletal/visceral assignment: one-half/one-half
- Coronal sectioning of rabbit fetuses
- Evaluation of cartilage

Added guidance on dermal and inhalation dosing

Statement included to indicate that treatment may be extended to include the entire period of gestation

Fetal evaluation is to be conducted without knowledge of treatment group

The section on evaluation of internal brain structure via midcoronal incision was reworded for clarity

Allows other categorization systems for fetal anomalies other than variations and malformations

B. Major changes to the reproductive toxicity test guideline:**Changes to mating procedures:**

- 2 weeks or 3 periods of estrous
- No opportunity to remate

Estrous cyclicity data:

- 3 weeks prior to mating
- During mating
- At termination

Sperm measures:

- Total number
- Percent progressively motile
- Morphology

Developmental milestones:

- Vaginal opening
- Preputial separation
- Anogenital distance (triggered in F2)

Adult postmortem:

- Selected organ weights
- Quantification of primordial oocytes

Weanling postmortem (one pup/sex/litter):

- Gross pathology
- Selected organ weights
- Histopathology if treatment-related effects

Increased number of pups in standardized litters (4 or 5/sex)

Optional termination of females at same stage of estrus is no longer specified

Sperm measures section was revised:

- Specifies that other forms of recording images are acceptable
- Provides better description of progressive motility
- Allows evaluation of number and morphology from recorded image
- Includes spermatid count

Adult histopathology section was revised:

- Detailed description of histopathological evaluation of testis and epididymis
- Revised criteria for sectioning ovaries and evaluating primordial oocyte population

Weanling postmortem section was revised:

- Gross necropsy of at least 3 weanlings/sex/litter (F1 and F2)
- Most organ weight requirements were deleted; those remaining are:
- Brain weight

Spleen and thymus

Fixation of grossly abnormal tissue and target organs, when known (F1 and F2)

Histopathological characterization of developmental anomalies, with special emphasis on reproductive organs

C. Changes to the adult histopathology section of the reproductive toxicity guideline:

Endpoints specified:

| | |
|-----------------------------------|---|
| Testis | Retained spermatids Missing germ cell layers or types Multi-nucleated giant cells Sloughing of spermatogenic cells into the lumen |
| Epididymis (caput, corpus, cauda) | Sperm granulomas Leukocytic infiltration (inflammation) Aberrant cell types within the lumen Absence of clear cells in the cauda epididymal epithelium |

| | |
|-------|--|
| Ovary | Presence or absence of corpora lutea of lactation Presence or absence of growing follicles Quantification of primordial follicle population from 5 sections, at least 100 microns apart, from the inner one-third, of each ovary. |
|-------|--|